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Humic Acid, Protein Hydrolysate & Microorganisms as a Mixed Consortium in Plant Growth

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Abstract: In recent decades, many farmers are using chemical fertilizers to cultivate the crop for fast growth, which culminates in an unhealthy and chemically contaminated food material. Instead of that, the humic acid, groundnut oil cake, protein hydrolysate and microorganisms were used as a biofertilizer to improve the crop production without any side effects. Three groups of 6 pots and one control were prepared by filling the above ingredients in different concentrations with green gram and black gram seeds to check the plant growth and plant height is measured and compared with other pots. Microbial density in soil was examined for this study. Level of protein hydrolysate is estimated by Lowry's method. Finally each ingredients were stimulated the plant growth and crop production. While the green gram showed good growth in low concentrated ingredients filled pot [S6(a)], the black gram showed good growth in medium concentrated ingredients filled pot [S6(b)]. This suggest that, mixed consortium of this ingredients are effective for the improvement of growth of green gram (Vigna radiate) and black gram (Vigna mungo) and for stimulating the increase of microorganisms in soil.

Key words: Humic acid, Biofertilizer, Protein hydrolysate, plant growth

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Farmers have adapted the strategy of increasing crop yield by applying large amount of chemical bio-fertilizers. Consequently, the negative effects of heavy use of chemical bio-fertilizers resulted in human health disorders, inferior quality of yield and adverse environmental conditions (Mishra *et al.*, 2011). Therefore, the ultimate goal of sustainable agriculture is to develop farming systems with high yields, profit and conservation of energy and natural resources such as soil and water, which would ensures food safety and quality (Winget & Gold, 2007).

For instance microbial fertilizers facilitate increase yield and quality of crops with appropriate investment of money and labor. It can clean the environment, generate plant nutrients like nitrogen, phosphorus, potassium through their activities in the soil or rhizosphere and make available to plants in a gradual manner (Muraleedharan *et al.*, 2010).

The fertilizers and bio-fertilizers are different from each other. Some may not be able to produce or supplement a specific nutrient to the plant. However plant nutrients are very essential for the production of crops and healthy food for the worlds expanding population (Chen, 2006).

Naggar (2006) suggested that the Nitrogen (N) is very important for the plant growth and to the crop production. But nitrogen fertilizers are available in small quantities at high prices as means of supplementing the soil or plant during growth. For supplementing plant nutrient like Nitrogen rich bio-fertilizers, Jingura (2010) used *Jatropha* oil cake to produce protein hydrolysate. In this regard Govindaraju (2003) worked with soy bean, ground nut flour and oil cake to estimate the amount of protein present by using enzymatic hydrolysis method. Ashwanikumar *et al.*, (2008) concluded that *Jatropha* oil cake can be used to produce a high yield of crop. Therefore, ground nut protein can also be used as a plant nutrient and packing materials to the microorganisms at low prices.

All the bio-fertilizers do not have capacity to hold the water and stimulate the dead cells but humic acid helps to break up the compacted soils and assists in transferring micronutrients to the plants, enhances water retention and increases seed germination (Stevention, 1994). Humic acid is referred to the combination of humic and fulvic acid content naturally occurring in the soil and agricultural waste deposits (Vallini *et al*., 1997). It can stimulate development of micro flora in soil and has the ability to stimulate the dead cells (Wang *et al*., 1995). Humic acid has the ability to hold seven times their volume in water than soil clays & it provides a carrier medium for nutrients required by soil organisms and plant roots (Pettit, 2004).

Therefore the purpose of this work is to observe the “Effects of humic acid, microorganisms and the protein hydrolysate from ground nut oil cake on the growth of green gram, fenugreek, black gram plant and on the microbial density in the soil.

Material and Methods

Soil samples were collected in sterile polythene bags from the three different agricultural fields such as paddy, ground nut and banana fields. All samples were serially diluted and the microorganisms were isolated and identified by standard procedure.

Production and estimation of protein hydrolysate

Protein hydrolysate production was carried out as per the procedure of Pittom (1914). In this procedure the ground nut (oil) cake was ground to powder. Then it was suspended in the 10% papine enzyme solution (10ml of papine enzyme mixed with 90ml of distilled water).Then it was kept in stirrer for 1 hour at 60^0C. Then the protein hydrolysate was controlled at temperature 100^0C in water bath for 20 minutes. After that, the supernatant (protein hydrolysate) was separated by centrifugation at 8000rpm for 10 minutes. The maximum yield of protein hydrolysate was estimated by Lowery et al (1951) method.

Optimization of Plant Growth in Pots

Artificial soil was prepared for this study. It was sterilized at 180^0C for 1 hour. Twenty five cm diameter pots were filled with compressed 150 gram of sterilized soil to within 1cm of the top. Soil in all pots was damp but not saturated. Pots were divided into 3 groups of 6 pots each. One pot was the negative control with only water added throughout the study. The percentage of concentrations was calculated based on the soil taken. The 3 sets of pots named as $S_1 - S_6$ and each set contains 6 pots and its concentrations were 1%, 2% & 3%respectively. In 3 pots of S1 Ground nut oil cake were added in the amount of 1.5g, 3gm & 4.5gm according to their concentration 1%, 2% & 3% respectively. Likewise it is followed for other set of pots S_2-S_5 (namely humic acid, isolated Bacterial culture, Protein Hydrolysate & Nitrogen fertilizer).

S6 pots were divided as S6(a), S6(b) & S6(c) according to the concentration and the amount of ingredients added in the pot. In this particular pot the amount of ingredients taken varies because it's all mixed together. In S_6(a) the concentration 1% and the amount of ingredients are 0.35gm of groundnut oil cake, o.35ml of bacterial culture (isolated 3 strains of bacterial culture was diluted in water), 0.35ml of Protein hydrolysate and 0.35gm of humic acid were added. In S6(b) concentration was 2% and the amount of ingredients are 0.75gm of groundnut oil cake, 0.75ml of bacterial culture 0.75ml of Protein hydrolysate and 0.75gm of humic acid was added. In S6(c) the concentration was 3% and the amount of ingredients were 1.12gm of groundnut oil cake, 1.12ml of bacterial culture, 1.12ml of Protein hydrolysate and 1.12gm of humic acid was added.

Black gram (Vigna mungo) and green gram (Vigna radiate) seeds were added in all pots and growth rate (Stem length in cm) was measured by using the scale.

Enumeration of Colony Forming Unit before and after inoculation of soil sample

The microbial density within the soil was measured on the day adding the culture to the pots and after 7 days of growth of the plants as per the method of Winget & Gold (2007)

Antibacterial activity of Humic Acid

Antimicrobial activity was estimated for humic acid by disc preparation kept in Luria bertani medium separated with microorganisms, such as *Staphylococcus aureus* (ATCC 29213), *Salmonella typhimurium* (ATCC 29629), *Escherichia coli* (ATCC 25922), *Clostridium perfringens* (ATCC 3626) and isolated soil organisms B1, B2 & B3 strains and kept in incubator

at 37^0C for 24 hours. This method was done to prove that humic acid can stimulate the activity of microorganisms**.**

Result:

Three bacterial strains were isolated from three rhizospheric soil samples and identified by standard methods as Micrococcus sp, Pseudomonas sp & Azotobacter sp only upto genus level.

Estimation of Protein in Protein hydrolysate prepared from Groundnut Oil cake:

Protein concentration (in %) of ground nut oil cake (Lowry's Method, 1951) was estimated as 57%.

Optimization of plant growth Rate in Green Gram

In green gram (S6 pot) the growth was high in 1% pot (S6a) concentration. It showed less growth rate in 2% (S6b) & 3 % (S6c) concentration. (Table 1; Fig 1)

Table 1: Showing the effect of growth of green gram in different growth substitutes
(Plant Stem in cm within 7 days)

Particulars/Concentrations	**1% Conc**	**2% Conc**	**3% Conc**
Control	4cm	4cm	4cm
ground nut oil cake (S1)	4.2cm	4.3cm	4.5cm
Humic acid (S2)	5.3cm	5cm	5cm
Bacterial culture (S3)	5.1cm	4.7cm	3.9cm
Protein hydrolysate (S4)	6cm	6.2cm	6.3cm
fertilizer (S5)	4.2cm	5.3cm	4.2cm
GOC+HA+B+Prtn Hydro (S6)	9cm (S6a)	8.2cm (S6b)	4.2cm (S6c)

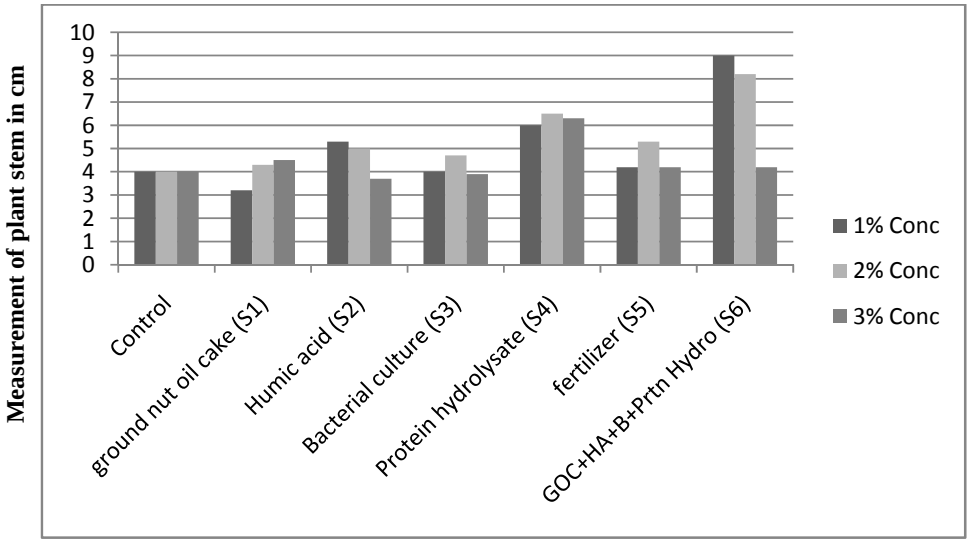

Fig 1: Showing High growth rate in 1% concentration of prepared biofertilizer

Optimization of plant Growth Rate in Black Gram

Black gram showed high growth rate in 2% concentration in S6 pot (S6b) and it showed less growth rate in 2% (S6a) & 3% (S6b) concentrations (Table 2; Fig 2)

Table 2: Showing the effect of growth of black gram in different growth substitutes

(Plant Stem in cm within 7 days)

Particulars/Concentrations	**1% Conc**	**2% Conc**	**3% Conc**
Control	3 cm	3cm	3cm
ground nut oil cake (S1)	4cm	4.2m	4.9cm
Humic acid (S2)	5.5cm	5.9cm	4.2cm
Bacterial culture (S3)	4.5cm	4.3cm	4cm
Protein hydrolysate (S4)	5.6cm	5.9cm	6cm
fertilizer (S5)	4cm	3.9cm	3.7cm
GOC+HA+B+Prtn Hydro (S6)	7.1cm	7.9cm	6.2cm

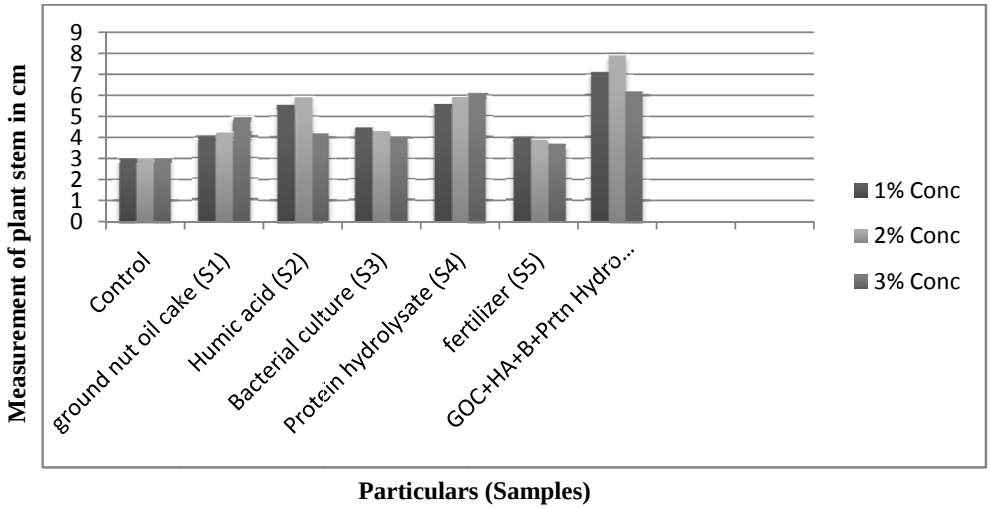

Fig 2: Showing High growth rate in 2% concentration of prepared biofertilizer

Enumeration of CFU before and after inoculation of soil samples

The Microbial density of the added microorganisms were gradually increases their growth.

Antimicrobial activity of humic acid

There is no zone of inhibition in the Luria Bertani medium containing microorganisms indicating that the humic acid is not an antimicrobial agent.

Discussion:

The isolated microorganisms like Micrococcus spp., Azotobacteria Spp., and Pseudomonas spp., are Phosphate Solubilizing Bacteria. These three bacterial strains are free living bacteria it can fix atmospheric nitrogen in cereal crops without any damage to the environment (Chen 2006). Due to the release of plant growth promoting substances by these organisms they increase the plant growth. Also converts the protein hydrolysate prepared from ground nut oil cake to Nitrogen source for the plant growth.

The ground nut oil cake contain minimum of 36-50% of crude protein (Naggar, 2006; Rahman & Ideresit, 2011). Jingura et al (2010) said that Jatropha oil cake contain Proteins and Carbohydrates. Moreover Jatrobha oil cake is used in industry for preparation of green manure and fertilizers, food, soap, pesticides, charcoal and medicinal uses (Ashwani kumar & Satyawati Sharma, 2008). Sumitra et al (2007) said that ground nut oil cake contain protein that can be used as a protein supplement for media preparation. Likewise, this study showed that the protein hydrolysate prepared from Ground nut oil cake increases the plant growth. And this protein hydrolysate can also be used as a packing material of this biofertilizer, because it supplementing

the food as nitrogen for both plant and microorganisms. Therefore the plant grows well in the pot containing only ground nut oil cake indicating that these plants can grow well with or without protein hydrolysate and microorganisms etc. At the same time addition of ground nut oil cake extract and its protein hydrolysate in increased concentration facilitates the growth of plants.

In this study humic acid was used to stimulate the plant growth and microorganisms present in the soil. This was proved by antimicrobial activity testing of Humic Acid and Enumeration of CFU from the pots which contain humic acid. The addition of humic acid to the both plant resulting in increase of plant nutrient uptake. In black gram the large amount (3%) of humic acid containing pots ($S_2 - S_5$) showed less plant growth when compared to the other concentrations 1% and 2% of humic acid containing pots. But it shows good result in medium concentration (2%) of humic acid containing pots ($S_2 - S_5$). Likewise, in Green Gram lower amount of humic acid containing pot S_6 (a) at 1% concentration shows high growth rate when compared to medium concentration (2%) and high concentration (3%) of humic acid containing pots. Because, excess amount of humic acid can destroy or decrease the microbial density in the soil (Fagbernro et al 1993). For this reason the microbial density of all pots containing humic acid and bacterial culture were carried out. It shows gradually increase their microbial density in lower concentration and decreasing result in higher concentration of humic acid.

Finally in this study, green gram showed good growth in the S_6 (a) sample where the ingredients are added in the lower concentration (1%) and it consists of ground nut oil cake, protein hydrolysate, Bacterial culture & humic acid. It shows all the ingredients are valuable and friendly with each other. Lower growth rate in S_6 (b) and S6(c) (ie., 2% & 3% concentration of this biofertilizer).

Likewise, In black gram it showed good growth in the S_6(b) sample where the ingredients are added in the medium concentration (2%) it consist of ground nut oil cake, protein hydrolysate, Bacterial culture & humic acid. Lower growth rate in S_6 (a) & S_6(c) (ie. 1% & 3% concentration of this biofertilizer).

Conclusion:

Although all the ingredients are useful and stimulate the plant growth, adding microorganisms and nutrient will facilitate the plant growth better when compared to chemical fertilizer. Ground nut oil cake and humic acid prepared from decomposed plant materials can improved the growth of plants. Therefore, the farmers can apply these ingredients for good production which are economically cheap and better for plant growth. Moreover, these ingredients are ecofriendly and helps in improving the soil fertility. Using biofertilizers we can keep the agricultural land unpolluted and helps in producing healthy products with high yield.

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